CLAIMS

1) A method of preparing T-cells for immunotherapy comprising

(a) Modifying T-cells by inactivating at least:

- A first gene expressing a target for an immunosuppressive agent, and

- A second gene encoding a component of the T-cell receptor (TCR)

(b) Expanding said cells, optionally in presence of said immunosuppressive agent.

2) The method according to claim 1 for preparing T-cells for immunotherapy, comprising the following steps:

(a) Providing a T-cell;

(b) Selecting a gene in said T-cell expressing a target for an immunosuppressive agent;

(c) introducing into said T-cell a rare-cutting endonucleases able to selectively inactivate by DNA cleavage respectively:

- said gene encoding a target for said immunosuppressive agent and

- at least one gene encoding one component of the T-cell receptor (TCR)

(d) Expanding said cells, optionally in presence of said immunosuppressive agent;

3) The method according to claim 1 or 2, wherein said target for an immunosuppressive agent is a receptor for an immunosuppressive agent.

4) The method according to any one of claims 1 to 3, wherein said transformed T-cells are expanded in patient's blood.

5) The method according to any one of claims 1 to 4, wherein said transformed T-cells are expanded *in-vivo*.

6) The method according to any one of claims 1 to 5, wherein said transformed T-cells are expanded in presence of said immunosuppressive agent.

7) The method according to any one of claims 1 to 6, wherein said gene expresses a target for an immunosuppressive agent which is CD52, and said immunosuppressive agent is an antibody targeting CD52 antigen.

8) The method according to any one of claims 1 to 6, wherein said gene expresses a glucocorticoid receptor (GR) and said immunosuppressive agent specific is a corticosteroid, such as dexamethasone,

9) The method according to any one of claims 1 to 6, wherein the at least two inactivated genes are selected from the group consisting of CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta.

10) The method according to any one of claims 2 to 9, wherein said rare-cutting endonucleases are co-transfected in step c).

11) The method according to any one of claims 2 to 10, wherein said rare-cutting endonucleases are encoded by mRNA.

12) The method according to any one of claims 2 to 11, wherein said rare-cutting endonucleases are introduced into said cell in step (c) by way of RNA electroporation.

13) The method of claim 12 comprising in step (c) contacting said T cell with RNA encoding rare-cutting endonuclease and applying agile pulse sequence consisting of:

(a) one electrical pulse with a voltage range from 2250 to 3000 V per centimeter, a pulse width of 0.1 ms and a pulse interval of 0.2 to 10 ms between the electrical pulses of steps (a) and (b);

(b) one electrical pulse with a voltage range from 2250 to 3000 V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and

(c) 4 electrical pulses with a voltage of 325 V with a pulse width of 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses.

14) The method according to any one of claims 2 to 13, wherein said rare-cutting endonucleases are TALE-nucleases.

15) The method according to claim 14, wherein at least one of these TALE-nuclease is directed against one of the gene target sequences of TCRalpha selected from SEQ ID NO: 37, SEQ ID NO: 57 to SEQ ID NO: 60.

16) The method according to claim 14, wherein at least one of these TALE-nuclease is directed against one of the gene target sequences of TCRbeta selected from SEQ ID NO: 38 and SEQ ID NO: 39.

17) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of GR selected from SEQ ID NO:1 to SEQ ID NO:6

18) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of CD52 selected from SEQ ID NO: 40, SEQ ID NO: 61 to SEQ ID NO: 65.

19) The method according to any one of claims 1 to 18, comprising introducing into said T-cells a Chimeric Antigen Receptor (CAR).

20) The method of claim 19 wherein said chimeric Antigen receptor sequence is SEQ ID NO: 73.

21) The method of claim 19 wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

22) The method according to any one of claims 1 to 21 comprising introducing into said T-cell a pTalpha polypeptide or a functional variant thereof.

23) The method according to any one of claims 1 to 22 comprising introducing into said T-cell a TALE-nuclease able to selectively inactivate by DNA cleavage PDCD1 or CTLA-4 gene.

24) The method according to any one of claims 1 to 23, wherein said T-cells in step a) are derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

25) The method according to any one of claims 1 to 23, wherein said T-cells in step a) are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.

26) An isolated T-cell or cell line obtainable from the method of any one of claims 1 to 25.

27) An isolated T-cell, in which at least two genes selected from the group consisting of: CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta, have been inactivated.

28) An isolated T-cell according to claim 26 or 27, further comprising an exogenous polynucleotide sequence encoding a Chimeric Antigen Receptor.

29) An isolated T-cell of claim 28 wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

30) An isolated T-cell according to any one of claims 26 to 29, further comprising an exogenous nucleic acid comprising at least a fragment of pTalpha transgene to support CD3 surface expression.

31) An isolated T-cell according to any one of claims 26 to 30 for its use as a medicament.

32) An isolated T-cell according to any of claims 26 to 30 for treating a cancer or a viral infection.

33) An isolated T-cell according to any of claims 26 to 30 for treating lymphoma.

34) A pharmaceutical composition comprising at least one isolated T-cell according to any one of claims 26 to 33.

35) A method for treating a patient in need thereof comprising:

(a) Preparing a population of T-cells according to the method of any one of claims 1 to 25;

(b)Administrating said transformed T-cells to said patient.

36) The method according to claim 35 wherein said patient is being treated with said immunosuppressive agent used in the method of claims 1 to 25.

37) The method according to claim 35 or 36, wherein said patient is diagnosed with cancer, viral infection, autoimmune disorders or Graft versus Host Disease (GvHD).

38) A TALE-nuclease directed against one of the selected target sequences of the TCR alpha gene selected from SEQ ID NO: 37 and SEQ ID NO: 57 to SEQ ID NO: 60.

39) A TALE-nuclease directed against one of the selected target sequences of the TCR beta gene SEQ ID NO: 38 and SEQ ID NO: 39.

40) A TALE-nuclease directed against one of the selected target sequences of the CD52 gene selected from: SEQ ID NO: 40 and SEQ ID NO: 61 to SEQ ID NO: 65.

41) A TALE-nuclease directed against one of the selected target sequences of the GR gene selected from: SEQ ID NO:1 to SEQ ID NO:6.

42) A TALE-nuclease according to claims 28 to 41 comprising one of the polypeptide sequence selected from SEQ ID NO: 7 to SEQ ID NO: 18 and SEQ ID NO: 41 to SEQ ID NO: 48.

43) A polynucleotide comprising a polynucleotide sequence encoding a TALE-nuclease according to any one of claims 38 to 42.

44) An isolated T-cell comprising at least one polynucleotide according to claim 43.

45) An isolated T-cell comprising at least two polynucleotides, said polynucleotides encoding at least a first and second TALENs, the first TALEN being directed against a gene encoding TCR and the second being directed against a gene encoding a target for an immunosuppressive agent.

46) An isolated T-cell according to claim 45, wherein said second TALEN is directed against a gene encoding CD52 or GR.

47) An isolated T-cell according to any one of claims 44 to 46, wherein it further comprises a polynucleotide sequence encoding a Chimeric Antigen Receptor.

48) An isolated T-cell according to any one of claims of 44 to 47, wherein it further comprises a polynucleotide comprising at least a fragment of pTalpha to support CD3 surface expression.

CLAIMS

1) A method of preparing T-cells for immunotherapy comprising

(a) Modifying T-cells by inactivating at least:

- A first gene expressing a target for an immunosuppressive agent, and

- A second gene encoding a component of the T-cell receptor (TCR)

(b) Expanding said cells, optionally in presence of said immunosuppressive agent.

2) The method according to claim 1 for preparing T-cells for immunotherapy, comprising the following steps:

(a) Providing a T-cell;

(b) Selecting a gene in said T-cell expressing a target for an immunosuppressive agent;

(c) introducing into said T-cell a rare-cutting endonucleases able to selectively inactivate by DNA cleavage respectively:

- said gene encoding a target for said immunosuppressive agent and
- at least one gene encoding one component of the T-cell receptor (TCR)

(d) Expanding said cells, optionally in presence of said immunosuppressive agent.

3) The method according to claim 1 or 2, wherein said target for an immunosuppressive agent is a receptor for an immunosuppressive agent.

4) The method according to any one of claims 1 to 3, wherein said transformed T-cells are expanded in presence of said immunosuppressive agent.

5) The method according to any one of claims 1 to 4, wherein said gene expresses a target for an immunosuppressive agent which is CD52, and said immunosuppressive agent is an antibody targeting CD52 antigen.

6) The method according to any one of claims 1 to 4, wherein said gene expresses a glucocorticoid receptor (GR) and said immunosuppressive agent specific is a corticosteroid, such as dexamethasone.

7) The method according to any one of claims 1 to 4, wherein the at least two inactivated genes are selected from the group consisting of CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta.

8) The method according to any one of claims 2 to 7, wherein said rare-cutting endonucleases are co-transfected in step c).

9) The method according to any one of claims 2 to 8, wherein said rare-cutting endonucleases are encoded by mRNA.

10) The method according to any one of claims 2 to 9, wherein said rare-cutting endonucleases are introduced into said cell in step (c) by way of RNA electroporation.

11) The method according to any one of claims 2 to 10, wherein said rare-cutting endonucleases are TALE-nucleases.

12) The method according to claim 11, wherein at least one of these TALE-nuclease is directed against one of the gene target sequences of TCRalpha selected from SEQ ID NO: 37, SEQ ID NO: 57 to SEQ ID NO: 60.

13) The method according to claim 11, wherein at least one of these TALE-nuclease is directed against one of the gene target sequences of TCRbeta selected from SEQ ID NO: 38 and SEQ ID NO: 39.

14) The method according to claim 11, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of GR selected from SEQ ID NO:1 to SEQ ID NO:6

15) The method according to claim 11, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of CD52 selected from SEQ ID NO: 40, SEQ ID NO: 61 to SEQ ID NO: 65.

16) The method according to any one of claims 1 to 15, comprising introducing into said T-cells a Chimeric Antigen Receptor (CAR).

17) The method of claim 16 wherein said chimeric Antigen receptor sequence is SEQ ID NO: 73.

18) The method of claim 16 wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

19) The method according to any one of claims 1 to 18 comprising introducing into said T-cell a TALE-nuclease able to selectively inactivate by DNA cleavage PDCD1 or CTLA-4 gene.

20) The method according to any one of claims 1 to 19, wherein said T-cells in step a) are derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

21) The method according to any one of claims 1 to 19, wherein said T-cells in step a) are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.

22) An isolated T-cell or cell line obtainable from the method of any one of claims 1 to 21.

23) An isolated T-cell, in which at least two genes selected from the group consisting of: CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta, have been inactivated.

24) An isolated T-cell according to claim 22 or 23, further comprising an exogenous polynucleotide sequence encoding a Chimeric Antigen Receptor.

25) An isolated T-cell of claim 24 wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

26) An isolated T-cell according to any one of claims 22 to 25 for its use as a medicament.

27) An isolated T-cell according to any of claims 22 to 25 for treating a cancer or a viral infection.

28) An isolated T-cell according to any of claims 22 to 25 for treating lymphoma.

29) A pharmaceutical composition comprising at least one isolated T-cell according to any one of claims 22 to 28.

30) A TALE-nuclease directed against one of the selected target sequences of the TCR alpha gene selected from SEQ ID NO: 37 and SEQ ID NO: 57 to SEQ ID NO: 60.

31) A TALE-nuclease directed against one of the selected target sequences of the TCR beta gene SEQ ID NO: 38 and SEQ ID NO: 39.

32) A TALE-nuclease directed against one of the selected target sequences of the CD52 gene selected from: SEQ ID NO: 40 and SEQ ID NO: 61 to SEQ ID NO: 65.

33) A TALE-nuclease directed against one of the selected target sequences of the GR gene selected from: SEQ ID NO:1 to SEQ ID NO:6.

34) A TALE-nuclease according to claims 30 to 33 comprising one of the polypeptide sequence selected from SEQ ID NO: 7 to SEQ ID NO: 18 and SEQ ID NO: 41 to SEQ ID NO: 48.

35) A polynucleotide comprising a polynucleotide sequence encoding a TALE-nuclease according to any one of claims 30 to 38.

36) An isolated T-cell comprising at least one polynucleotide according to claim 35.

37) An isolated T-cell comprising at least two polynucleotides, said polynucleotides encoding at least a first and second TALENs, the first TALEN being directed against a gene encoding TCR and the second being directed against a gene encoding a target for an immunosuppressive agent.

38) An isolated T-cell according to claim 37, wherein said second TALEN is directed against a gene encoding CD52 or GR.